Patterning of the vertebrate embryo along the left–right (L/R) axis is required for proper positioning and asymmetric development of the visceral organs. The conserved role of the Nodal signaling pathway during this process has been well established, but how asymmetric gene expression is interpreted by tissues to result in asymmetric morphogenesis is still not well understood. To address this question, we have studied the processes of cardiac jogging and looping in zebrafish. We find that Nodal signaling influences the direction of myocardial migration within the cardiac cone, just prior to jogging, and that the direction of these cellular movements are reversed in mutants with defects in asymmetric gene expression. In addition, we find that this event results in a repositioning of the original L/R axis to the dorsal–ventral (D/V) axis of the linear heart tube. Finally, we have discovered the existence of a rotation within the heart tube just prior to cardiac looping which converts the D/V axis back to the L/R axis. While the direction of this rotation is reversed in morphants with defects in asymmetric gene expression, the reestablishment of the original L/R axis occurs properly, regardless of Nodal signaling laterality. These results suggest a role for asymmetric gene expression in directing the first axis conversion during cardiac jogging but indicate that the second axis conversion at the initiation of cardiac looping may occur in a Nodal-independent manner.

doi:10.1016/j.ydbio.2008.05.438

Program/Abstract # 458

3-*O*-sulfotransferase is required for cardiac development and physiology in zebrafish

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Heart development involves precise coordination of patterning events, cell movements and cell physiology in order to generate a functional heart. Cell surface and extracellular heparan sulfate proteoglycans (HSPGs) are core proteins with modified glycosaminoglycan (GAG) chains that are thought to mediate interactions between cells and their environments. We have cloned multiple 3-*O*-sulfotransferases (3-OSTs)1 presumed to add sulfate to the 3-position carbon of GAGs in HS, and are systematically assessing their roles in development. Morpholino knockdown of one of the 3-OST family members, 3-OST-7, results in a hypoplastic cardiac ventricle that does not contract properly, resulting in poor blood circulation and pericardial edema. What is the underlying mechanism for the observed cardiac ventricular defect? Primary heart field specification and patterning, and development of the vasculature and atrioventricular valve appear to be normal. In contrast, the outflow tract fails to form properly in morphant embryos. Moreover, action potential and intracellular calcium measurements indicate that ventricular contraction is uncoupled from excitation. Together these results indicate that 3-OST-7 has multiple roles in heart development, and that 3-OST function might provide a novel mechanism for the regulation of cardiac cell physiology. 1. Cadwallader AB, Yost HJ. Combinatorial expression patterns of heparan sulfate sulfotransferases in zebrafish: I. The 3-*O*-sulfotransferase family. Dev Dyn. 2006;235:3423-31.

doi:10.1016/j.ydbio.2008.05.439

Program/Abstract # 459

Channel independent functions of L-type calcium channel beta-2 subunit

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Calcium channel beta-2 (CACNB2) subunits regulate voltage-gated channel electrophysiological dynamics and chaperon newly synthesized pore-forming alpha subunits to the plasma membrane. In addition to these canonical roles as calcium channel modulators, recent studies indicate that the beta subunits may have channel-independent functions that relate to their MAGUK (Membrane Associated Guanylate Kinase) protein structure. MAGUK family proteins perform a variety of scaffolding functions in the cell. We report the discovery of two CACNB2 genes in zebrafish. We find that the cardiac cells of CACNB2 morpholino-treated embryos dissociate more easily under pressure, and are reduced in number. To determine if cardiac myocyte morphology and cell adhesion is compromised in beta-2 morphants, we assayed cardiac cellular organization with several membrane markers including N-Cadherin.

doi:10.1016/j.ydbio.2008.05.440

Program/Abstract # 460

Tbx5-mediated β2 CaMK-II expression is required for heart looping and pectoral fin development

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Mutations in the gene encoding Tbx5, result in Holt–Oram syndrome (HOS), which is characterized by defective cardiac development and stunted forelimbs. The genetic targets of Tbx5 responsible for proper heart and limb development are still being identified. In zebrafish embryos, the functional suppression of type II Ca^{b+}/calmodulin dependent protein kinase (CaMK-II) results in both aberrant cardiac looping and diminished pectoral fin development similar to tbx5 morphant and mutant (*heartstrings: hst*) embryos. Morphants of just one of the seven genes encoding catalytically active CaMK-II in early zebrafish embryos (β2 CaMK-II; *camk2b2*) exhibit the *hst* phenotype. *Camk2b2* mRNAs are transiently expressed in the heart and limb buds at the time of heart looping. Cardiac abnormalities in *camk2b2* and *tbx5* morphants can be reversed by overexpression of cytosolic CaMK-II. Normal fin development can be restored by CaMK-II in *camk2b2* morphants, but not in *tbx5* morphants. Both *tbx5* morphant and *hst* embryos exhibit diminished β2 CaMK-II, while the introduction of excess Tbx5 into zebrafish embryos and mouse fibroblasts increases β CaMK-II expression. Tbx5 also promotes transcription of a *camk2b2*-reporter, most likely through direct interaction with the evolutionarily conserved Tbx5 binding elements found in β CaMK-II genes. These findings indicate that Tbx5 induces β CaMK-II expression, which is necessary for normal cardiac and limb development.

doi:10.1016/j.ydbio.2008.05.441

Program/Abstract # 461

Hedgehog signaling plays a cell-autonomous role in maximizing cardiac developmental potential

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